## LARVAL TREMATODES FROM AUSTRALIAN TERRESTRIAL AND FRESHWATER MOLLUSCS

## PART IV CERCARIA (FURCOCERCARIA) MURRAYENSIS n. sp.

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Cercaria murrayensis was one of the commonest larval trematodes present in Limnaea lessoni gathered from the River Murray near Tailem Bend. In May, 1937, fourteen of 119 specimens gave off these cercariae in the aquarium; in June only five of these snails, all uninfected, were obtained; in early December eight out of 135 were found infected; in March, 1938, eight out of 41; and in April six out of 48. In early December, during an excursion to Swan Reach, River Murray, under the auspices of the newly-formed Tate Society, we collected 439 specimens of the Limnaea, 200 of which gave off these cercariae in the aquarium.

The parasites could be recognised casily when the tube containing them was held against the light, the greater number maintaining a characteristic resting position (fig. A), suspended in the water. In this the tail stem was usually perpendicular and the two furcae were separated by an angle of about 120°. The proximal part of the tail stem was bent and in line with the body, making a considerable angle with the rest of the tail. By far the greater number of cercariae were motionless at any one time, and the resting period usually varied between five and thirty seconds. This inactivity was broken by short bursts of movement, and the cercaria would move rapidly tail foremost (fig. B) twisting itself spirally and varying the rate of speed. As the greater number were hanging with the tail stem more or less perpendicular, the general movement was upwards, but could occur in any direction, the latter sometimes changing.

In measuring cercariae, the method outlined by Cort and Brackett (1937) was followed and the material killed by adding to it an equal volume of boiling 10% formalin. Specimens were examined and the following are the measurements of 30: length of body  $138 \mu$  -  $185 \mu$ , average  $158 \mu$ ; breadth of body across ventral sucker 32-46  $\mu$ , average  $37 \mu$ ; anterior tip of body to centre of ventral sucker 77-131  $\mu$ , average  $100 \mu$ ; length of tail stem  $161-208 \mu_{\pi}$  average  $188 \mu$ ; width of tail stem  $24-34 \mu$ , average  $27\cdot5 \mu$ ; length of furcae  $154-200 \mu$ , average  $173 \mu$ ; length of anterior organ  $45-60 \mu$ , average  $54 \mu$ ; length of posterior sucker  $22-29 \mu$ , average  $25\cdot9 \mu$ ; breadth of posterior sucker  $22-27\cdot5 \mu$ , average  $25\cdot7 \mu$ .

The body was finely corrugated, and thus it was extremely difficult to see the body spines clearly. The large anterior organ showed no differentiation into two parts, and the well developed ventral sucker lay just behind the middle of

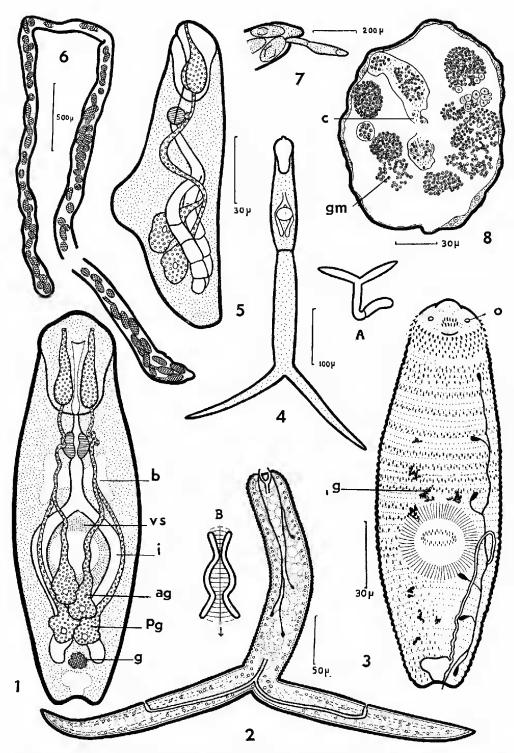


Fig. A, cercaria in resting position; B, cercaria moving; 1, body of cercaria; 3, tail; 3, excretory system, spination; 4, entire cercaria; 5, lateral view of cercaria; 6, portions of a sporocyst; 7, cercaria emerging from sporocyst; 8, T.S. sporocyst.

Figs. 1, 3 drawn to same scale

the body. Yellow pigment granules were present scattered throughout the body, and concentrated into two groups lying near the junction of the intestinal caeca.

About twelve large forwardly directed spines occurred on the highly contractile anterior tip in front of the mouth (fig. 3). Surrounding this region was a flattened spineless area on which opened the mouth and the ducts of the gland cells. This was succeeded by a collar of spines of varying sizes arranged somewhat irregularly in from five to seven rows, with the largest spines in front. Another spineless area separated these from the much smaller spines of the general body surface. These latter, larger on the ventral than on the dorsal surface, were arranged irregularly just below the collar, with a tendency to form rows on the ventral surface, and behind this they were grouped in nine double rows, the last being on a level with the middle of the ventral sucker. The spines posterior to this were arranged irregularly on the ventral surface, and were much more numerous at the posterior end near the tail. Two irregular rows were present on the ventral sucker. We were unable to ascertain whether there were any minute spines on the tail stem, but they appeared to be present on the furcae.

The mouth was subterminal, the pre-pharynx short and the pharynx well developed. The oesophagus bifurcated just in front of the ventral sucker into the well-defined caeca, which were characteristically bent and reached almost to the bladder. They were filled with a clear refracting substance which was not continuous but separated into masses, so that the intestine appeared at first sight to be composed of a few large cells. It stained well with neutral red.

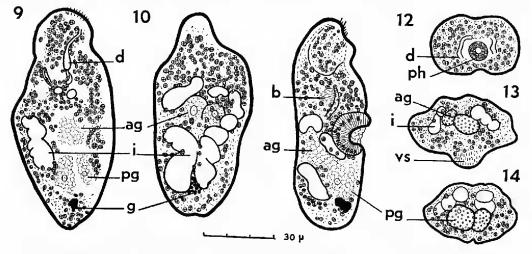
The four gland cells (figs. 1, 5) stainable with nile blue sulphate lay behind the acetabulum, ventral to the caeca. They were coarsely granular and slightly lobed with large clear nuclei, and arranged in two pairs, those in the first pair being somewhat tandem, and those in the second opposite. The ducts passed forwards in two pairs, following the course indicated, and in all but fully extended specimens were twisted in the region of the pharynx. After entering the anterior organ they became much enlarged, then narrowed before opening dorso-laterally on the circumoral spineless area.

The genital primordium consisted of a mass of undifferentiated cells lying between the ends of the caeca and the bladder. The nervous system was represented by an H-shaped mass of tissue lying posterior to the pharynx. Longitudinal and circular muscle fibres were present in the tail stem, and there were about forty caudal bodies of varying size with a tendency to become arranged in indefinite groups.

From each of the antero-lateral borders of the small bladder, mushroom-shaped when fully distended, arose a main excretory duct. Each was slightly coiled and proceeded upwards and ontwards to about the middle of acetabulum, where it doubled back and, on a level with the posterior margin of the ventral sucker, gave rise to an ascending and descending branch. The flame cell formula was  $2 \times (6 + 2) = 16$ . Of the three flame cells connected with the anterior tubule, one lay alongside the anterior organ, the second near the pharynx, and

the third just in front of the acetabulum. Of the three connected with the descending branch, one was just posterior to the ventral sucker, the second about midway between this and the posterior end, and the third alongside the bladder. The descending tubules passed into the tail stem, each bearing two flame cells. The island of Cort was small, and the main excretory tube passed down the centre of the tail stem to divide into two just before reaching the furcae. Each branch opened on the edge of the furca (fig. 2) about half-way along its length.

The sporocysts present in tangled masses in the liver were very hard to separate. They were attenuated, one end being pointed with a birth pore just behind the tip, and the other end bluntly rounded. They contained numerous germ balls and developing cercariae. Older sporocysts had a thin wall formed of cuticle and sparse, flattened epithelial cells (fig. 8), the latter being several layers deep at the end of the sacs. Germ masses and mature germ cells were typical.



Figs. 9, 10, consecutive longitudinal horizontal sections of cercaria; 11, longitudinal section of cercaria; 12, 13, 14, Tr. sections through a cercaria

Cort and Brackett (1937a) gave a brief resumé of the Strigeid cercariae obtained from Douglas Lake, Michigan, and of these our specimen resembles most closely C. flexicauda and C. yogena. The behaviour in free life of our species was almost identical with that of the former (Cort and Brooks, 1928), and its average lengths of body, tail stem and furcae respectively were  $158 \mu$ ,  $188 \mu$ ,  $173 \mu$ , compared with  $170 \mu$ ,  $254 \mu$  and  $226 \mu$  in C. flexicauda, and  $173 \mu$ ,  $236 \mu$  and  $221 \mu$  in C. yogena. Our species was considerably smaller than both the American forms and the furcae and tail stem both approximated more nearly to the length of the body. The spination resembled most closely that of C. yogena, and the pigmentation characteristic of the American form was almost identical with ours, though as yet no pigmentation has been seen in the tail stem. The caudal bodies and excretory system resembled those of C. flexicauda, while differing from those of C. yogena. There was no ciliation of the main collecting tubes as in the latter

and the position of the flame cells differed. The anterior organ was the same length as in C. flexicauda, but the ventral sucker was smaller, being  $26 \mu$  long in our form and  $35 \mu$  in the American.

Cort and Brackett (1937 b) published a paper on the identification of Strigeid corcariae, utilizing differences in their behaviour during free life. Before receiving their article we had already noticed such behaviour in our specimens, and were able to distinguish the species with the naked eye from amongst a collection of corcariae.

Wesenberg-Lund (1934) drew attention to that group of Strigeid cercariae characterised by the presence of four penetration gland cells behind the ventral sucker, which had been mentioned by Cort and Brooks (1928). To this *Proalaria* group of pharyngeal, longifurcate, distome cercariae of Miller (1926) our species belongs, and appears to us to be a typical member, having for its intermediate stage a *Diplostomulum* present in the eyes of certain freshwater fish.

We suggest the name *Cercaria murrayensis* for this *Proalaria* larva and propose to give an account of experimental infections of various fish in a later paper.

## REFERENCES

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## EXPLANATION OF FIGURES

All drawings were made with the aid of the camera lucida, except figs. A and B, and the details of fig. 3. Figs. drawn to scale indicated.

Ag, anterior gland cell; b, brain; c, cercaria; d, duct of gland cell; g, genital rudiment; gm, germ mass; i, intestine; o, opening of ducts of gland cells; pg, posterior gland cell; ph, pharynx; vs, ventral sucker; yg, yellow granules.